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Genetics

Construction of a yeast strain devoid of mitochondrial introns and its use to screen nuclear genes involved in mitochondrial splicing

(intron-less mitochondria/self-splicing/*Saccharomyces cerevisiae*/*pet*⁻ mutations)

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ABSTRACT **We have constructed a respiring yeast strain devoid of mitochondrial introns to screen nuclear *pet*⁻ mutants for those that play a direct role in mitochondrial intron excision. Intron-less mitochondria are introduced by cytoduction into *pet*⁻ strains that have been made *rho*⁰; cytoductants therefrom recover respiratory competency if the original *pet*⁻ mutation is required only for mitochondrial splicing. By this means, we have identified 11 complementation groups of such genes. Their total number may be estimated as about 18.**

In *Saccharomyces cerevisiae* the genes of the 21S rRNA, cytochrome *b* (*cob*), and subunit 1 of the cytochrome *c* oxidase (*cox1*) have a mosaic structure (1). Ten of the 13 mitochondrial introns contain an open reading frame whose product, called maturase (2), is involved in splicing its host intron. The reading frame of eight of these maturases is in phase with that of the upstream exon so they are synthesized as hybrid proteins. Their translation is therefore dependent on the translation of the gene bearing them. As a consequence, any mutation affecting specifically the translation of such a gene will appear as a mutation affecting the splicing, although it is merely a secondary effect of the block on translation.

It is clear that numerous nuclear-encoded gene products are necessary for the expression of mitochondrial genes. Among nuclear *PET* genes (3), which are involved in post-transcriptional steps of mitochondrial gene expression, some are necessary for the excision of introns (4–7) whereas others are implicated in the translation of specific mitochondrial mRNA (8–10). In the present work, we describe a genetic test enabling discrimination of the former from the latter. The rationale of this test consists of the fact that a nuclear gene whose only essential function is the excision of one or several introns becomes dispensable in a strain from which this or these introns have been withdrawn. Therefore, if intron-less mitochondria are introduced by cytoduction (11, 12) into a *pet*⁻ strain that has been made *rho*⁰, cytoductants thereof will recover respiratory competency if and only if the original *pet*⁻ mutation affects the mitochondrial splicing exclusively. To screen this class of *pet*⁻ mutations, we have built a fully respiratory competent strain devoid of mitochondrial introns. We have estimated as about 18 the number of nuclear genes that intervene directly in the splicing of mitochondrial introns. This indicates that even though mitochondrial introns appear to be endowed with autocatalytic properties (13) a large number of nuclear or mitochondrial proteins participate in intron excision processes.

MATERIALS AND METHODS

Media. Complete solid media (1% yeast extract/1% Bacto-peptone/2% Bacto-agar) were supplemented with either 2% glucose (YPD), 2% glycerol (YPG), or 2% glycerol and 0.1% glucose (YPdif). Minimal medium [0.68% yeast nitrogen base/1% glucose/2% Bacto-agar (W)] was supplemented with 0.05% yeast extract/0.05% Bacto-peptone (WR) for replica crosses. YPD + ethidium bromide: YPD medium containing 50 mM phosphate buffer (pH 6.25) and ethidium bromide (50 μg/ml). YPD10: YPD with 10% glucose. AA medium: solid aminoadipate medium containing 0.167% yeast nitrogen base without ammonium sulfate, 10% glucose, 2% Bacto-agar, 0.2% α-DL-aminoadipic acid, 40 mg of L-lysine and L-methionine per liter (14). CAN medium: 0.67% yeast nitrogen base/10% glucose/2% Bacto-agar/40 mg each of L-canavanine, L-lysine, and L-methionine per liter.

Yeast Strains. A list of strains used is given in Table 1. D273-10B/G1 and D273-10B/U17 were spontaneous *lys2* and *ura3* mutants of strain D273-10B/A (22) selected according to Chattoo *et al.* (14) and Boeke *et al.* (23), respectively. D273-10B/G1 cells were γ-irradiated from a ^{60}Co source (5 krad) and then mixed with D273-10B/U17 cells (24). Diploid clones were isolated and forced to sporulate, and one of the resulting haploids, D273-10B/E1B, was used further. The mitochondrial genome of D273-10B/G1 was destroyed (*rho*⁰) by ethidium bromide treatment (25), and then a spontaneous canavanine resistant mutant, D273-G10/F11, was isolated. The mitochondria of M12-54 were introduced into D273-G10/F11 by cytoduction (11, 12). WR210/cyb10 is a *rho*⁻ clone derived from WR210, a respiratory competent revertant of WR200. The *rho*⁻ mutagenesis was done according to Fukuhara and Rabinowitz (26). The mitochondrial genome of WR210/cyb10 is 6.5 kilobases (kb) long and contains a *cob* gene with no intron (27).

Selection of *mit*⁻ Mitochondrial Mutants. Yeast strains were treated with 7 mM $MnCl_2$ (28, 29). Dilutions of the mutagenized cells were spread onto YPdif plates to give ≈200 colonies per plate. Small colonies were plated onto YPD plates. These master plates were replica-plated onto YPG (to test whether the clones were effectively *gly*⁻) and replica-crossed onto WR plates with each of the *rho*⁻ testers CEO2, CEP2, or O_1P2 (17). After 2 days of incubation, the latter plates were replica-plated onto YPG, and growth was scored after 3 days of incubation. As mentioned (17), this series of crosses allows the identification of *mit*⁻ mutations among *gly*⁻ mutants and roughly localizes them on the mitochondrial genome. *mit*⁻ in the *cox1* region were more precisely mapped using the *rho*⁻ testers HD1, M513, and DS6-A407.

***In Vivo* Deletion of Mitochondrial Introns.** Respiratory competent revertants of *mit*⁻ mutants localized in the *cox1* or *cob* genes were searched for as follows. Five culture tubes each containing 3 ml of YPD were inoculated with aliquots of the *mit*⁻ mutant of interest. After 24 hr of incubation, the cultures were centrifuged and cell pellets were resuspended in 0.1 ml of 0.9% NaCl and then plated onto YPG. *Gly*⁺ revertants appeared after 5–14 days. Each *gly*⁺ revertant was subcloned on a YPG plate. Total yeast DNA of individual subclones was extracted

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Table 1. Yeast strains

Strains	Genotype	Source
D273-10B/A	*MATα met6* [D273]	A. Tzagoloff
D273-10B/G1	*MATα met6 lys2* [D273]	This work
D273-10B/G1/2	*MATα met6 lys2* [rho^0]	EtdBr treatment of D273-10B/G1
D273-10B/U17	*MATα met6 ura3* [D273]	This work
D273-10B/E1B	*MATa met6 ura3* [D273]	This work
D273-10B/E1B/F11	*MATa met6 ura3* [rho^0]	EtdBr treatment of D273-10B/E1B
D273-G10/F11	*MATα met6 lys2* can^R [rho^0]	This work
K5/2	*MATa Kar1-1 trp5* cyh^R [rho^0]	Séraphin *et al.* (15)
M12-54	*MATa ilv5 trp2* [M12-54]	Kutzeb *et al.* (16)
GF107-3A	*MATα met6* lys2 [M12-54]	Meiosis of D273-10B/G1/2 × M12-54
Kar(M12-1)	*MATa Kar1-1 trp5* cyh^R [M12-54]	Cytoductant of GF107-3A × K5/2
D273-M121	*MATα met6 lys2* can^R [M12-54]	Cytoductant of D273-G10/F11 × Kar(M12-1)
D273-M3A	*MATα met6 lys2* can^R [M12-54]	Meiosis of D273-M121 × D273-10B/E1B/F11
D273-M16C	*MATa met6 ura3* can^R [M12-54]	Meiosis of D273-M121 × D273-10B/E1B/F11
MH41-7B/HF21	*MATa ade2 his1* [rho^0]	H. Fukuhara
MH41-7B/M726	*MATa ade2 his1* [rho^-]	(CEO2) Bolotin-Fukuhara *et al.* (17)
MH41-7B/L721	*MATa ade2 his1* [rho^-]	(O_1P2) Bolotin-Fukuhara *et al.* (17)
MH41-7B/AB83	*MATa ade2 his1* [rho^-]	(CEP2) Bolotin-Fukuhara *et al.* (17)
HD1	*MATa ura* [rho^-]	R. Schweyen
M513	*MATa his4* [rho^-]	G. Carignani
DS6-A407	*MATα* [rho^-]	G. Macino
KAR1-1	*MATa Kar1-1 his3 ino1 ino4* [rho^0]	E. Sena (18)
WR200	*MATa his4* [777-3A, ΔΣbI]	gly^-, P. Pajot
WR210	*MATa his4* [777-3A, ΔΣbI]	Revertant gly^+ from WR200
WR210/cyb10	*MATa his4* [rho^-]	EtdBr treatment of WR210
NCYC74	*MATa*/*MATα* [NCYC74]	*S. uvarum*, ATCC 24904
UVA32	*MATα* [NCYC74]	Meiosis of NCYC74 [ten Berge (19)]
Kar(UVA32)	*MATa Kar1-1 trp5* cyh^R [NCYC74]	Cytoductant of K5/2 × UVA32
D273-G13	*MATα met6 lys2* can^R [NCYC74]	Cytoductant of Kar(UVA32) × D273-G10/F11
GF157-4B	*MATα lys2* can^R [NCYC74, ΔΣbI]	Meiosis of D273-G13 × WR210/cyb10
GF155-1B	*MATa lys2 his4* can^R [NCYC74, ΔΣbI]	Meiosis of D273-G13 × WR210/cyb10
GF157-4B/1012-R1	*MATα lys2* can^R [NCYC74, ΔΣbI, ΔaI5γ]	This work
D273-10B/G1/224	*MATα met6 lys2* [D273, ΔaI5γ]	This work
D273-10B/G1/356-R5	*MATα met6 lys2* [D273, ΔaI1,2,3]	This work
GF113-4A	*MATa ade2 his1* [D273, ΔaI5γ]	Meiosis of MH41-7B/HF21 × D273-10B/G1/224
GF113-4A/18C	*MATa ade2 his1* [rho^-]	EtdBr treatment of GF113-4A
GF137-13A	*MATa his1 met6* [D273, ΔaI1,2,3,5γ]	Meiosis of GF113-4A/18C × D273-10B/G1/356-R5
GF134-6D	*MATα his1 met6* [D273, ΔaI1,2,3,5γ]	Meiosis of GF113-4A/18C × D273-10B/G1/356/R5
GF106-11D	*MATa ade2* [D273, ΔaI1,2,3]	Meiosis of MH41-7B/HF21 × D273-10B/G1/356-R5
GF106-11D/20-2A	*MATa ade2* [rho^-]	EtdBr treatment of GF106-11D
GF167-7B	*MATα lys2* [NCYC74, ΔΣbI, ΔΣaI]	Meiosis of GF157-4B/1012-R1 [mit^-] × GF106-11D/20-2A
Kar(4B1)	*MATa Kar1-1 trp5* cyh^R [NCYC74, ΔΣbI]	Cytoductant of K5/2 × GF157-4B
Kar(134)	*MATa Kar1-1 trp5* cyh^R [D273, ΔaI1,2,3,5γ]	Cytoductant of K5/2 × GF134-6D
Kar(167)	*MATa Kar1-1 trp5* cyh^R [NCYC74, ΔΣbI, ΔΣaI]	Cytoductant of K5/2 × GF167-7B

The mitochondrial genomes are indicated in brackets. The original mitochondrial genomes of D273-10B/A, M12-54, 777-3A, and NCYC74 contain the following sets of introns. [D273]: ω, aI1, aI2, aI3, aI4, aI5γ, bI4, bI5; [M12-54]: aI1, aI2, aI3, aI4, aI5α, aI5β, aI5γ, bI1, bI2, bI3, bI4, bI5; [777-3A]: same set as [M12-54] plus ω; [NCYC74]: aI2, aI3, aI5γ, bI4, bI5. Introns bI1, aI1, aI2, and aI5γ belong to group II; the others belong to group I (20, 21). ω, 21S rRNA intron; aI, cytochrome oxidase subunit 1 intron; bI, cytochrome b intron. Δ, Intron deletions. ΔΣbI and ΔΣaI indicate that all the introns of *cob* or *cox1* are deleted. Mitochondria with different intron combinations were introduced into K5/2 cells by cytoduction. EtdBr, ethidium bromide.

using a rapid procedure (30), digested with the enzymes *Eco*RI and *Bam*HI, and then electrophoresed on a 0.7% agarose gel. DNA was visualized after ethidium bromide staining and the positions of mitochondrial DNA bands were compared to those of the parental strain. Revertants resulting from deletion of mitochondrial introns (31) were readily identified by this method. We obtained a single deletion each of intron aI5γ, aI3, bI5 and the simultaneous deletion of aI1 and aI2 or of aI1, aI2, and aI3 from strain D273-10B/G1.

Selection of *pet*⁻ Nuclear Mutants. Yeast strains were treated with ethyl methanesulfonate as described (29). gly^- mutants were screened on YPdif plates. Recessive pet^- mutants were distinguished from mit^- or rho^- by crossing gly^- mutants with a rho^0 strain: *pet* [rho^+] × *PET* [rho^0] gives respiratory competent diploids.

Genetic Screening of *pet*⁻ Mutations Impeding Mitochondrial Splicing. pet^- mutants were first plated onto YPD plates (50–55 colonies per plate). These master plates were then replica-plated onto a series of different plates according to the protocol schematized in Fig. 1.

RNA Transfers. RNA was extracted from purified mitochondria as described (32). RNA transfers to nitrocellulose filters and RNA·DNA hybridization were performed according to the Southern method as modified by Thomas (33).

RESULTS AND DISCUSSION

Whenever a yeast nuclear gene is involved in splicing mitochondrial introns, its inactivation (by a mutation) produces respiratory deficiency in a strain containing a full set of introns, it is without effect in a strain lacking all of them, provided the gene has no other essential function. With such an intron-less strain being available, we can devise a genetic

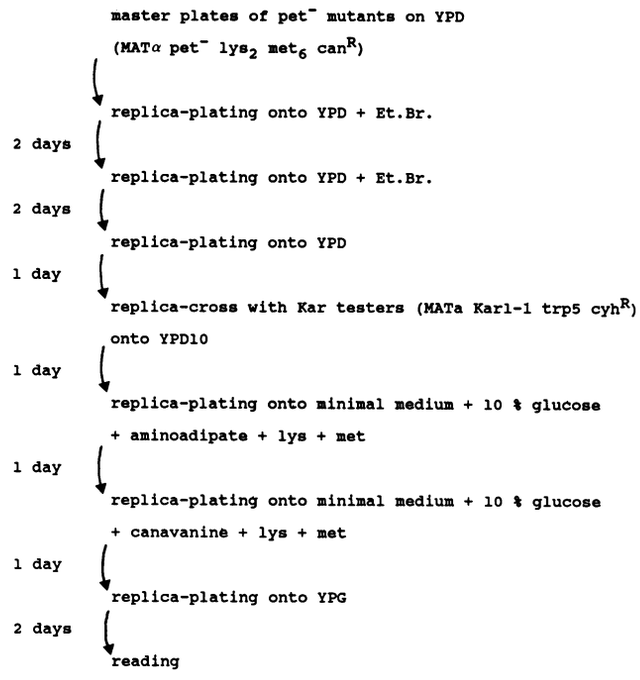

FIG. 1. Protocol for screening *pet*⁻ mutations impeding mitochondrial splicing. The parental strain D273-M3A was plated on each plate as a control. The ethidium bromide (Et.Br.) treatment eliminates the mitochondrial genome (*rho*⁰ cells). The *pet*⁻ [*rho*⁰] cells were replica-crossed with each of the strains K5/2, Kar(M12-1), Kar(4B1), Kar(134), and Kar(167). Plating onto aminoadipate and canavanine media allowed the selection of cytoductants carrying the *pet*⁻ nucleus from Kar cells and diploid cells.

test to screen those *pet*⁻ nuclear mutations acting directly on mitochondrial splicing. The ideal way to carry out this screening is to prepare a series of *pet*⁻ mutants from a strain possessing the 13 mitochondrial introns [such as KL14-4A or 777-3A (1)], then to examine the effect of these mutations in the same strain cleared of its mitochondrial introns. However, we decided to isolate *pet*⁻ mutants from a strain bearing the mitochondria of M12-54, in which the ribosomal intron ω is absent. The reason for this choice is that defective splicing of the rRNA intron by any mutation will impede the assembly of mitoribosomes and such mutants will degenerate into *rho*⁻ or *rho*⁰ (34). Potential *pet* mutations involved in splicing all group I introns (35) may then escape detection.

Construction of a Mitochondrial Genome with No Intron. To build such a mitochondrial genome, we took advantage of the following facts: (*i*) the mitochondrial genome of strain NCYC74 contains only five introns [aI2, aI3,aI5γ, bI4, bI5 (1)], (*ii*) strains devoid of introns in cytochrome *b* have already been isolated (27, 36) and one of them was used to prepare a *rho*⁻ mutant, WR210/cyb10, containing the intron-free *cob* gene (see *Materials and Methods*). D273-G13 and WR210/cyb10 were crossed, and *rho*⁺ genomes with the intron-free *cob* gene were formed. After meiosis, a haploid strain, GF157-4B, was obtained. It still contained introns aI2, aI3, and aI5γ. *mit*⁻ mutations were generated in the aI5γ region of GF157-4B and a revertant that had lost intron aI5γ, GF157-4B/1012-R1, was recovered. *mit*⁻ mutations localized in the aE1–aE2–aI2 region of this latter strain were isolated. One of them, crossed with *rho*⁻ GF106-11D/20-2A, whose parental strain possessed the mitochondrial genome of D273-10B/G1/356-R5, allowed us to obtain *gly*⁺ diploid recombinants lacking aI2 and aI3. In their intron-free mitochondrial genomes, ≈18,000 base pairs are deleted with respect to 777-3A. Nevertheless, the sporulation of these diploids was quite normal, as was the germination of the spores therefrom. Strain GF167-7B is a haploid derived from one of these diploids.

We compared the growth rate of the two isonuclear strains Kar(UVA32) and Kar(167) carrying the mitochondria of NCYC74 and GF167-7B, respectively, in YPD, YPG, and lactate liquid media. Growth rates of both strains were identical (data not shown). This demonstrates that yeast mitochondrial introns are not necessary to confer respiratory competency. However, they may be advantageous in special environmental conditions or could be implicated in some functions pertaining to recombination or DNA repair for instance (37, 38), although both strains present a similar proportion of spontaneous *gly*⁻ mutations after an overnight culture in YPD10 (≈0.4%).

Screening of *pet*⁻ Mutants Involved in Mitochondrial Splicing. We prepared a collection of *pet*⁻ mutants by treating the isonuclear and isomitochondrial strains D273-M3A and D273-M16C with ethyl methanesulfonate. Approximately 300 and 250 *pet*⁻ derived, respectively, from D273-M3A and D273-M16C were isolated. We attempted to gain a rough estimate of the number of complementation groups represented by the 300 *pet*⁻ issued from D273-M3A. These latter 300 *pet*⁻ were crossed with the 250 *pet*⁻ derived from D273-M16C. By this means, 64 complementation groups were distinguished but 137 *pet*⁻ isolated from D273-M3A and 116 *pet*⁻ from D273-M16C could not be included in these groups. Forty-one of the 137 *pet*⁻ were chosen at random, their mating type was changed through meiosis, and they were crossed to each other (41 × 41 crosses). Thirty-six complementation groups were then found. So, we estimate that the 137 *pet*⁻ belong to at most 120 complementation groups (137 × 36/41). Hence, the 300 *pet*⁻ derived from D273-M3A should represent 64 + 120 = 184 complementation groups, although we are aware that this figure is overestimated.

One member from each of the 64 complementation groups and the 137 *pet*⁻ mutants (all of them derived from D273-M3A) were made *rho*⁰ by ethidium bromide treatment. These *rho*⁰ cells were crossed with K5/2, Kar(M12-1), Kar(4B1), Kar(134), and Kar(167) as described in *Materials and Methods*. Cytoductants carrying the nucleus of D273-M3A were recovered through two successive selection steps: resistance to α-aminoadipate and then resistance to canavanine. The respiratory competency of cytoductants was tested. The different steps of this screening procedure were essentially done by replica-plating, allowing simultaneous treatment of a large number of *pet*⁻ mutants. The *pet*⁻ [*rho*⁰] × K5/2 crosses were used as a control. When crossed with Kar(M12-1), the *pet*⁻ [*rho*⁰] recovered their original mitochondria; thus, the *lys2 can*^{R} cytoductants should be respiratory deficient. We retained those *pet*⁻ whose cytoductants were clearly *gly*⁻ when crossed with Kar(M12-1) and *gly*⁺ when crossed with Kar(134), Kar(4B1), or Kar(167). These comprised 19 *pet*⁻. All of them gave *gly*⁺ cytoductants when crossed with Kar(134), Kar(4B1), and Kar(167) except A124, A203, A226, and A256 whose cytoductants were *gly*⁻ when crossed with Kar(134). These 19 *pet*⁻ were subsequently crossed with M12-54, GF137-13A, or GF155-1B. Diploids thereof were forced to sporulate and spores were analyzed at random by selecting the *can*^{R} spores or, in some cases, tetrads were dissected. Results are presented in Table 2. The spore analyses from *pet*⁻ × M12-54 crosses suggest single gene segregations, whereas those from *pet*⁻ × GF137-13A or *pet*⁻ × GF155-1B crosses confirm the results obtained from the *pet*⁻ screening with strains Kar(134) and Kar(4B1), respectively.

We have determined the number of complementation groups formed by the 19 *pet*⁻ mutations identified as potentially affecting nuclear genes involved in the splicing of mitochondrial introns. *MATa pet*⁻ were obtained from the diploid *pet*⁻ [*rho*⁰] × M12-54 described above. The three *pet*⁻, *mss116*, *mss18*, and

Table 2. Meiotic analyses

	Testers					
	M12-54		GF137-13A		GF155-1B	
pet⁻	*can*^{R}*gly*⁻	*can*^{R}*gly*⁺	*can*^{R}*gly*⁻	*can*^{R}*gly*⁺	*can*^{R}*gly*⁻	*can*^{R}*gly*⁺
A18*	2 *gly*⁻	2 *gly*⁺ (4)	0 *gly*⁻	4 *gly*⁺ (4)		
A65†	2 *gly*⁻	2 *gly*⁺ (5)	0 *gly*⁻	4 *gly*⁺ (4)		
A98*	72	61	3	192		
A115	31	45	20	121		
A124‡	30	28	41	73	0 *gly*⁻	4 *gly*⁺ (4)
A128†	30	32	57	214		
A133	34	31	0	15		
A142	24	25	7	75		
A179	45	41	17	320		
A183*	39	52	0	183		
A190	154	205	13	144		
A203‡	179	167	21	23	0 *gly*⁺	4 *gly*⁺ (2)
A221*	27	32	2	34		
A226	24	32	28	23	14	95
A230*	43	67	17	119		
A253*	34	24	5	121		
A256‡	29	31	94	141	0	210
A278	25	20	34	181		
A286	35	45	1	71		

pet⁻ mutants were crossed with M12-54, GF137-13A, or GF155-1B on YPD plates. Diploids were replica-plated onto W and then forced to sporulate. Asci were digested with glusulase and either tetrads were analyzed or dilutions of sonicated asci were spread onto CAN plates. *can*^{R}*gly*⁺ and *can*^{R}*gly*⁻ colonies were identified by replica-plating onto YPG. The mitochondria of M12-54 and Kar(M12-1), GF137-13A and Kar(134), GF155-1B and Kar(4B1), respectively, are identical. Half of the *can*^{R} spores issued from *pet*⁻ × M12-54 crosses should be *gly*⁺ (i.e., 2 *gly*⁺/2 *gly*⁻ tetrads). The *pet*⁻ whose cytoductants were *gly*⁺ when crossed with Kar(134) should give rise to 100% *can*^{R}*gly*⁺ spores when crossed with GF137-13A; similarly, the *pet*⁻ whose cytoductants were *gly*⁺ when crossed with Kar(4B1) should give rise to 100% *can*^{R}*gly*⁺ spores (i.e., 0 *gly*⁻/4 *gly*⁺ tetrads) when crossed with GF155-1B. Numbers in parentheses indicate the number of tetrads analyzed. *, †, and ‡ indicate different members of three complementation groups.

mss8 (6), were also included in this test. Eleven complementation groups were found. Three groups are composed of 6, 3, and 2 elements, respectively (Table 2). *petA179* is allelic to *mss116*. These results prove the reliability of our test since we were able to screen independent allelic mutations. Preliminary investigations with Kar strains harboring the different combinations of intron deletions we have at our disposal suggest that *petA124* is necessary for the excision of bI5 (data not shown). The corresponding gene was isolated by genetic complementation. Its sequence is identical to that of the gene *CBP2* described by McGraw and Tzagoloff (39). The use of the same Kar strains showed that *petA179* is involved in the splicing of aI1 and perhaps in that of aI5α, aI5β, bI1, bI2, or bI3.

RNA Blots. Regardless of the intron content, M12-54 is not isomitochondrial to NCYC74 from which the intron-less mitochondrial genome was derived. Therefore, we may suspect that after substitution of their mitochondria, the recovery of respiratory competency in some *pet*⁻ mutants is not due to the absence of introns but could rather be accounted for by some special part of the mitochondrial genome of GF167-7B, which had no equivalent in M12-54 mitochondria. It is necessary to show that the isolated *pet* mutants are indeed defective in RNA splicing. This is demonstrated by RNA blot experiments. Mitochondria from isonuclear and isomitochondrial strains petA18, -A124, -A142, -A179, -A190, -A278, -A286, and from D273-M3A were purified, RNA extracted, and then electrophoresed on a 1.1% agarose gel, transferred to a nitrocellulose filter, and probed with plasmids pEx1-1 and pBE1 harboring part of the exon 1 sequence of *cox1* and *cob*, respectively. The autoradiograms are shown in Fig. 2. The results may be summarized as follows: petA18 blocks the splicing of the RNA of cox1, whereas cob RNA intermediates accumulate. The cox1 RNA pattern of petA124 is slightly disturbed (two bands are missing) and the cob mature RNA is greatly diminished and larger RNA species accumulate. Almost no cox1 RNA transcripts are detected in petA142, whereas the processing of cob RNA is normal; the effect of petA142 is similar to that of mss8 (6), although these two mutations are not allelic. If petA142 is actually involved in the splicing of cox1 RNA we have to suppose that some splicing defects may lead to RNA instability. The splicing of cox1 and cob RNAs is completely blocked in petA179. In petA190 the cox1 RNA pattern is quasi-normal (just one band seems to be missing), whereas the cob RNA pattern is normal. The splicing of cox1 RNA is blocked in petA278, but the processing of cob RNA is normal. The amount of cox1 and cob mature RNA is reduced in petA286 and cob splicing intermediates accumulate. In summary, petA18, -A124, -A179, -A278, and -A286 definitely appear to affect nuclear genes involved in splicing mitochondrial introns. With regard to

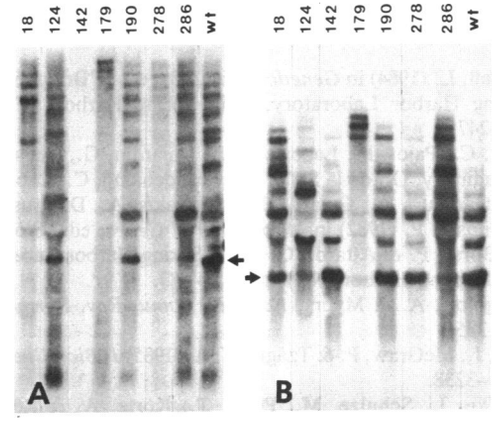

FIG. 2. RNA transfer blot analysis of the mitochondrial RNA of petA18, -A124, -A142, -A179, -A190, -A278, -A286, and D273-M3A. (*A*) The probe (pEx-1) was a *Dra* I/*Hha* I fragment from exon 1 of *cox1*. (*B*) The probe (pBE-1) was the *Nar* I/*Eco*RI fragment from exon 1 of *cob*. wt, D273-M3A. Arrows indicate position of mature mRNA.

petA142 and petA190, further studies are necessary to prove that they participate in this process.

Evaluation of the Number of Nuclear Genes Involved in Mitochondrial Splicing. As mentioned above, *pet*$^-$ isolated from D273-M3A should represent about 184 complementation groups (64 + 120). With the screening described herein, we found that 11 complementation groups of 184 are assumed to correspond to genes participating in RNA splicing of mitochondrial introns. Knowing that 300 *pet*$^-$ complementation groups have been so far described (40) and supposing that the 184 complementation groups constitute a representative sample of these 300 *pet*$^-$ complementation groups, we may estimate as about 18 (300 × 11/184) the number of nuclear genes involved in mitochondrial splicing. Note that potential *pet*$^-$ mutants specifically involved in rRNA splicing are not included in the present screening since a rRNA intron-less strain was used for *pet* mutagenesis.

Mitochondrial introns, either of group I or group II, possess conserved nucleotide sequences and similar secondary structures (20, 21), but since only some of them are self-spliced *in vitro* (7, 41, 42), we may then suspect that the other mitochondrial introns are not endowed with enough catalytic ability for their own excision. To help the excision processes, about 18 nuclear gene products and 9 mitochondrial maturases should intervene; we may then suppose that on the average two proteins—i.e., (18 + 9) proteins/13 introns—interact with each intron sequence (and presumably with both adjacent exon sequences) to sustain the catalytic properties of introns. In an alternative model, these proteins (at least the nuclear gene products) would be constituents of mitochondrial spliceosomes (43). It is also possible that some of the *PET* gene products are involved in the posttranslational maturation of maturases (2) and that some other ones regulate the expression of the nuclear genes participating directly in mitochondrial splicing.

Assuming that no more mitochondrial introns are discovered, the screen described in this report should allow one to isolate the majority of the nuclear genes involved in mitochondrial splicing, avoiding the tedious determination of complementation groups. However, if some of these genes are also involved in other cellular processes, or if they lead to a high rate of *rho*$^-$ and *rho*^{0} deletions, their discrimination would escape our test, unless conditional *pet*$^-$ mutants are used.

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